collected in tubes. Density is checked using spectrophotometic enumeration and McFarland nephelometer standards. Approximately 3x10/12/ml in Stock. Motility is checked with Motility agar slant (Northeast Laboratory Services). Stock is diluted to concentration of approx. $1x10^9$ per ml in PBS and stirred for 1 hr at room temperature. The Flagella is removed from the outside of the bacteria. Supernatant is collected using centrifugation. Pellet of whole bacteria is separated from the supernatant. Dry weight approx.14.7mg/ml is determined and the material is used as Stock Immunogen for H antigen. It is diluted to 1 mg/ml in PBS and heated for 30 minutes at 60-70 C. This helps keep contamination down to a minimum. Thiogylcollate broth is inoculated to check for growth and animals are inoculated with immunogen.

Example 4: Preparation of O Antigen for Immunogens

Brain Heart Infusion (BHI, acumedia) is used to stimulate the O antigens on the bacterium. Stock TSB innoculate BHI Broth is formed and incubated at 37°C for 18 hrs. This stimulates somatic antigen development on the bacteria. Flasks containing BHI Broth are inoculated with BHI Broth culture. While stirring slowly, flasks are incubated at 37°C. Good growth is seen after 22 hours. Flasks are combined and the material is harvested using centrifugation and sterile saline (0.9%) at approx. 3000rpm for 30 minutes. The harvest is collected in tubes. Density is checked using spectrophotometic enumeration and McFarland nephelometer standards. The material is diluted to approximately $1x10^9$ per ml. 4% sodium deoxycholate (Difco) solution is added as a 1:1 ratio with culture in 0.9% sterile saline (Herzberg, 1972) and stirred for approx. 18 hrs at room temperature (22 to 24C). The material is centrifuged to remove whole cells. Supernatant is used as stock for O antigen. Dry weight is determined at approximately 14.9mg/ml. the product is diluted in sterile PBS, pH 7.4 to 1mg/ml for O Immunogen.

grown under strict anaerobic conditions. The stock culture is grown according to ATCC for #12662. As with other organisms, subcultures are grown in small amounts. Thioglycollate Media (Difco) is inoculated with the stock and incubated for 48 hrs. Flasks are inoculated with Reinforced Clostridial Medium Broth. The medium is covered with a mixture of anaerobic gas. Flasks are combined and the product is harvested using centrifugation at approximately 2500 rpm for 30 minutes. The product is collected in tubes and spun at low speed for 30 minutes. Density is checked using spectrophotometric enumeration and McFarland nephelometer standards. The product is centrifuged to remove whole cells. The supernatant is used as stock for CS antigen. It is heated at 60°C for 40 min. to inactivate if needed. Dry weight is determined at approximately 22mg/ml. The product is diluted with PBS, pH 7.4 to 1mg/ml for CS Immunogen.

Example 9: Preparation of CA antigen for Immunogen

The Reinforced Clostridial Medium is used for CA Antigen Production. It is a standard medium for stimulating adherence antigens for *Clostridium aminophilius*. These cultures must be grown under strict anaerobic conditions. The stock culture is grown according to ATCC for #49906. As with other organisms, subcultures are grown in small amounts. Thioglycollate Media (Difco) is inoculated with the stock and incubated for 48 hrs. Flasks are inoculated with Reinforced Clostridial Medium Broth. The medium is covered with a mixture of anaerobic gas. Flasks are combined and the product is harvested using centrifugation at approx 2500 rpm for 30 minutes. The product is collected in tubes and spun at low speed for 30 minutes. Density is checked using spectrophotometer enumeration and McFarland nephelometer standards. The product is centrifuged to remove whole cells. The supernatant is used as stock for CA antigen. It is heated at 60°C for 40 min. to inactivate if needed. Dry weight is determined at

initial injection. If boosters were needed, 100ug was given in each booster (every 6 months). Within four weeks, 4 out of the 6 hens produced excellent antibodies in the eggs. ELISA WC readings averaged 0.95 OD for 1:10,000 dilution and 0.250OD for 1:50,000. After six weeks the average ELISA O reading was 0.95 OD for 1:20,000 dilution with still 5 chickens responding.

Example 16: Immunization of Chicken with A Immunogen:

Six selected egg laying hens, 6 White Leghorn, approximately 19 weeks old were injected with the stock A Immunogen. Four injections (500ug, 100ug, 200ug, and 250ug) were given 1 week apart. A serum sample was collected two weeks after the last initial injection. If boosters were needed, 100ug were given in each booster (every 6 months). Within four weeks, 5 out of the 6 hens produced excellent antibodies in the eggs. ELISA A readings averaged 1.40 OD for 1:10,000 dilution and 0.576 OD for 1:50,000. After six weeks the average ELISA A reading was 1.15 OD for 1:20,000 dilution with still all chickens responding.

Example 17: Immunization of Chicken with P Immunogen:

Six selected egg laying hens, White Leghorn , approximately 19 weeks old were injected with the stock P Immunogen. Four injections (500ug, 100ug, 200ug, and 250ug) were given 1 week apart. A serum sample was collected two weeks after the last initial injection. If boosters were needed, 100ug were given in each booster (every 6 months). Within four weeks, 5 out of the 6 hens produced excellent antibodies in the eggs.

Example 18: Immunization of Chicken with CS Immunogen:

Six selected egg laying hens, White Leghorn, approximately 19 weeks old were injected with the stock CS Immunogen. Four injections (500ug, 100ug, 200ug, and

250ug) were given 1 week apart. A serum sample was collected two weeks after the last initial injection. If boosters were needed, 100ug was given in each booster (every 6 months). Within four weeks, All 5 out of 6 hens produced excellent antibodies in the eggs.

Example 19: Immunization of Chicken with CA Immunogen:

Six selected egg laying hens, Red Sex-Linked Hybrids, approximately 19 weeks old were injected with the stock CA Immunogen. Four injections (500ug, 100ug, 200ug, and 250ug) were given 1 week apart. A serum sample was collected two weeks after the last initial injection. If boosters were needed, 100ug was given in each booster (every 6 months). Within four weeks, All 6 hens produced excellent antibodies in the eggs.

Example 20: Preparation of Stock Production Whole Egg reagents

Selected hens were combined from all four immunogen groups for E. coli O157:H7 or three immunogen groups for the anaerobes, to be used to produce production batches of whole egg reagents. Sterling U.S. Patent 5,753,228 presents an excellent review of uses for the selection of eggs and storage of same. The eggs were randomized and shell removed. The whole egg is mixed well and pasteurized using standard conditions (60°C (140° F) for 3.5min), Charley, H. and C. Weaver,3rd edition, Foods: a scientific approach, Merrill-Prentice Hall, p350, 1998). Once pasteurized, samples were tested for activity and stored at 4° C until dried or sprayed onto carriers. Samples of 250µl were analyzed.

Examples of results for ELISAs are given:

Pasteurized Whole Egg: E. coli O157:H7

Immunogen	Dilution	O.D.
WC	500	0.532
WC	2500	0.113
H	500	0.466
H	2500	0.115
O	500	0.338
O	2500	0.128
A	500	0.588
A	2500	0.155

Pasteurized Whole Egg: Anaerobes

Immunogen	Dilution	Batch #1	Batch#2	Batch #3
CA	100	0.339	0.275	0.627
CA	500	0.104	0.296	0.201
P	100	0.724	0.882	0.576
P	500	0.248	0.594	0.651
CS	100	0.457	0.268	0.650
CS	500	0.304	0.143	0.476

Example 21: Coating of Feed Additive Carriers

Although whole egg can be dispensed in water supplies, or in a dried format as whole powdered egg, use of a carrier helps distribute the material in a uniform method. This makes it easier for mixing with standard feeds. A number of carriers can be used to provide a vehicle as a feed additive as needed. Soy hulls in crude, refined and pelted format, rice hulls, corn, cottonseed hulls, distilled dried grains, beet pulp or any other. The production pasteurized whole egg prep is coated on to the carrier and either fed directly to the animals or dried to 10-15% moisture. Approximately 1000ml of whole, pasteurized egg is sprayed on 50lbs of pelleted soybean hulls. The preferred carrier for cattle is pelleted soybean hulls while for young swine the fines from the pelleted soybean hulls. The feed additive is mixed with the standard animal feed. The preferred level is 10-15lbs of feed additive to 2000lbs of animal feed.

Example 22: Analysis of Feed Additive samples after coating with reagents

Samples were collected from batches of Feed Additive after they were coated on to the carr. ers. The samples were analyzes and the results are as follows:

Product Name	Moisture %	Protein %	Fat %	Fiber, crude %
Crude Soybean Hulls, uncoated	11.59	26.76	9.10	18.63
CAMAS EYE 0157 Crude soybean Hulls	12.35	25.67	8.26	19.46
CAMAS EYE * Control Crude Soybean hulls	12.06	24.89	9.92	20.38
Soybean Pellets uncoated	11.65	9.89	2.43	33.47
CAMAS EYE Efficiency Pellets	12.37	10.19	2.57	33.12

* CAMAS EYE identifies inhibitors produced according to the present invention

Example 23: Analysis of Production Eggs over Time: E. coli O157:H7

Samples of the Whole Egg preparations were analyzed using the ELISA systems for H, O, WC and A immunogens to monitor activity over time after the initial immunization schedule was completed. Selected animals from each group were placed into the Production group. The average ELISA OD readings (Negative subtracted) for the fourth through the six months are given in the table below. The eggs were sampled using 250µl of the whole eggs and diluted 1:500 and 1:2500 in PBS buffer and then run in the appropriate ELISA to determine the average OD reading at each dilution. The negative control readings are subtracted from each reading. The immunogens showed different responses in the animals along with good specificity. The A immunogen gave the best responses in these tests. Data for these immunogens over time is given below:

Immuogen	Fourth Month	Fifth Month	Six Month
H : 1:500	0.388	0.848	0.718
1:2500	0.085	0.237	0.195
O: 1:500	0.593	0.792	0.704
1:2500	0.147	0.294	0.184
WC: 1:500	0.398	0.730	0.578
1:2500	0.062	0.273	0.130
A: 1:500	0.700	1.014	0.909
1:2500	0.102	0.305	0.224

Example 24: Analysis of Production Eggs over Time: Feed Efficiency

Samples of the Whole Egg preparations were analyzed using the ELISA systems for P, CS, and CA immunogens to monitor activity over time after the initial immunization schedule was completed. Selected animals from each group where placed into the Production group. The average ELISA OD readings for the fourth through the six months are given in the table below. The eggs were sampled using 250µl of the whole eggs and diluted 1:500 and 1:2500 in PBS buffer and then run in the appropriate ELISA to determine the average OD reading at each dilution. The negative control readings are subtracted from each reading. The immunogens showed different responses in the animals along with good specificity.

Immuogen	Fourth Month	Fifth Month	Six Month
P : 1:500	1.182OD	1.128OD	0.942OD
1:2500	0.785OD	0.489OD	0.343OD
CS: 1:500	0.843OD	0.989OD	0.582OD
1:2500	0.318OD	0.356OD	0.187OD
CA: 1:500	1.156OD	1.087OD	0.998OD
1:2500	0409OD	0.282OD	0.507OD

Example 25: Analysis of Feed Additives for Antibody Activity: *E. coli* O157:H7

Samples of the coated hulls were analyzed using the ELISA systems for H, O, WC and A immunogens to monitor activity after pasteurizing, spraying, drying and storage.

Good antibody response was recorded after the processing of the Production Whole Egg batches and drying on crude soybean hulls. Data for two batches is given below:

Batch: Coated Hulls	WC Immunogen	H Immuogen	O Immunogen	A immunogen
Batch #1 1:10	0.673 OD	1.103 OD	1.105 OD	1.299 OD
1:100	0.106 OD	0.236 OD	0.229 OD	0.302 OD
Batch #2 1:10	1.174 OD	1.291 OD	1.180 OD	1.224 OD
1:100	0.177 OD	0.396 OD	0.327 OD	0.458 OD

Example 26: Analysis of Feed Additives for Antibody Activity: Feed Efficiency

Samples of the coated hulls were analyzed using the ELISA systems for P,CS, and CA immunogens to monitor activity after pasteurizing, spraying, drying and storage. Good antibody response was recorded after the processing of the Production Whole Egg batches and drying on crude soybean hulls. One gram samples of the 15lbs of coated hulls was extracted and analyzed. Data for three batches is given in the table below:

Batch: Coated Hulls	P Immunogen	CS Immuogen	CA Immunogen
Batch #1 1:100	0.067OD	0.289OD	0.051OD
1:500	0.057OD	0.131OD	0.037OD
Batch #2 1:100	0.028OD	0.039OD	0.095OD
1:500	0.049OD	0.015OD	0.021OD
Batch#3 1:100	0.046OD	0.115OD	0.136OD
1:500	0.012OD	0.055OD	0.012OD

Example 27: Recovery of Active Antibody and Egg Protein After Feed Mix

Bags of coated soybean refined hulls were coated with the production whole egg reagent containing anti-E. coli O157:H7 adherence inhibitors. One bag of feed additive (15lbs) was added to 2000lbs of Standard Cattle Feed. Control Feed Additive was produced with whole eggs from free ranging chickens. Soybean hulls were coated with this preparation and mixed as the Test Feed Additive containing the specific antibodies. Samples of the mixed Feed were collected and analyzed for active antibody to the ELISA

WC immunogen as well as a commercial ELISA for detecting Egg Protein in food (Veratox® Quantitiative Egg Allergen Test, Neogen). The data is given in the chart below for two batches of feed ration.

Mixed Feed	First Batch	Second Batch
Test Feed-Additive: 1:6000 1:12000	0.172 OD 0.009OD	0.112OD 0.036
Control Feed-No Additive 1:6000 1:12000	0.049 0.005	Neg. Neg.
Test Feed-Additive: Egg Protein	0.958OD 17ppm	1.268OD >20ppm
Control Feed-No Additive: Egg Protein	0.800OD 15 ppm	1.050OD 20ppm

Example 28: Feeding of Cattle

Two groups of cattle were feed either the O157:H7 feed additive (coated onto refined soybean hulls) or control feed additive (coated with control eggs and no specific adherence inhibitors). The animals were feed at a rate of 15lbs of feed additive per 2000 lbs feed. They average 10lbs per animal per day. Animals weighed approximately 1000lbs when they started and over 1400 when sent to market. All animals looked very healthy with the Test animals eating more feed during the 87days. Five of the test animals were positive during the start of the experiment for E. coli O157:H7 and only one of the control animals. Within 30 days on feed additive all test animals were negative for E. coli O157:H7 and stayed negative for three consecutive samples over a 30 day period. Standard protocols were followed for sampling. All animals were ear-tagged and placed

in separate pens. Animals were sampled on a weekly basis for the first month and then bi-weekly after that until they were shipped to market. Grab samples were taken from the rectum and placed into sterile labeled bags. All samples were held on ice until processed in the lab. All samples were processed within 4 hours of collection each day. The fecal samples were diluted with TSB with 0.6% yeast extract. Dilutions of the mixture were streaked onto Sorbitol-MacConkey's agar with or without cefixime-tellurite supplement (Dynal®). Colorless colonies are picked for further testing. A latex agglutination test was used to identify E. coli serogroup O157 (Oxoid dry Spot™ E. coli 0157). If positive, then individual colonies were selected for further isolation on SMC agar streak plates. Isolated colonies were run on the commercial EIA for EH E. coli O157 (Binax, NOW® EH E. coli O157). Biochemical confirmation can be done with API-20E (Analytab Products). (Appl. Environ. Microbiol. 62(7)2567-2570, 1996, J. Clin. Micro. 36(10):3112, 1998)

One of the most startling and distressing characteristics of E. coli 0157:H7 is the small number of microorganisms necessary to produce cases of human illness. By way of example, at least 10,000 of the more virulent Salmonella serotypes but as few as ten E. coli 0157:H7 are required to cause a person to become symptomatic. Therefore, one animal hosting or externally contaminated with the microorganism can, when slaughtered, affect as much as 16 tons of ground beef to the extent that a single helping of the product could result in illness if improperly prepared. Although the probability of any one animal hosting the microorganism at any one time is low, the probability of its presence in any one particular feedlot is high.

There are presently three different methods for protecting the consumer from the E. coli 0157:H7 threat which have been officially recognized. The three methods are (1) thorough cooking, (2) steam pasteurization and (3) irradiation, all of which have specific drawbacks, including human and mechanical error, cost, consumer resistance, and the like.

Any microorganism which colonizes the alimentary tract of its host must possess the capability of sticking or adhering to that surface in order to multiply. E coli 0157:H7 is no exception to this rule. The adherence inhibitor of this invention strongly interferes with adherence and, on a cumulative basis, thereby prevents the specific targeted microorganism from colonizing and multiplying. Through the vehicle of a simple daily feed additive, the product essentially supplies the host with a specific antibody preparation designed not to cure any

disease in the animal (cattle are essentially unaffected by E. coli 0157:H7 being only transitory hosts) but merely to dislodge any resident bacteria and to prevent the attachment of any newly introduced bacteria in the alimentary tract. The adherence inhibitor has no direct effect on the host itself, leaves absolutely no undesirable residue in the animals and thus has no effect whatsoever on the ultimate food products. In addition, since the microorganism is prevented from multiplying, it will over time (for example the 120 day finishing period in the feedlot) disappear through natural degradation from the mud and manure coating the animal, eliminating this significant potential source of contamination at slaughter. Properly managed, the risk of cross contaminating other food sources through feedlot runoff or by the application of manure as fertilizer is also essentially eliminated.

It is apparent that many modifications and variations of this invention as hereinbefore set forth may be made without departing from the spirit and scope thereof. The specific embodiments described are given by way of example only and the invention is limited only by the terms of the appended claims.